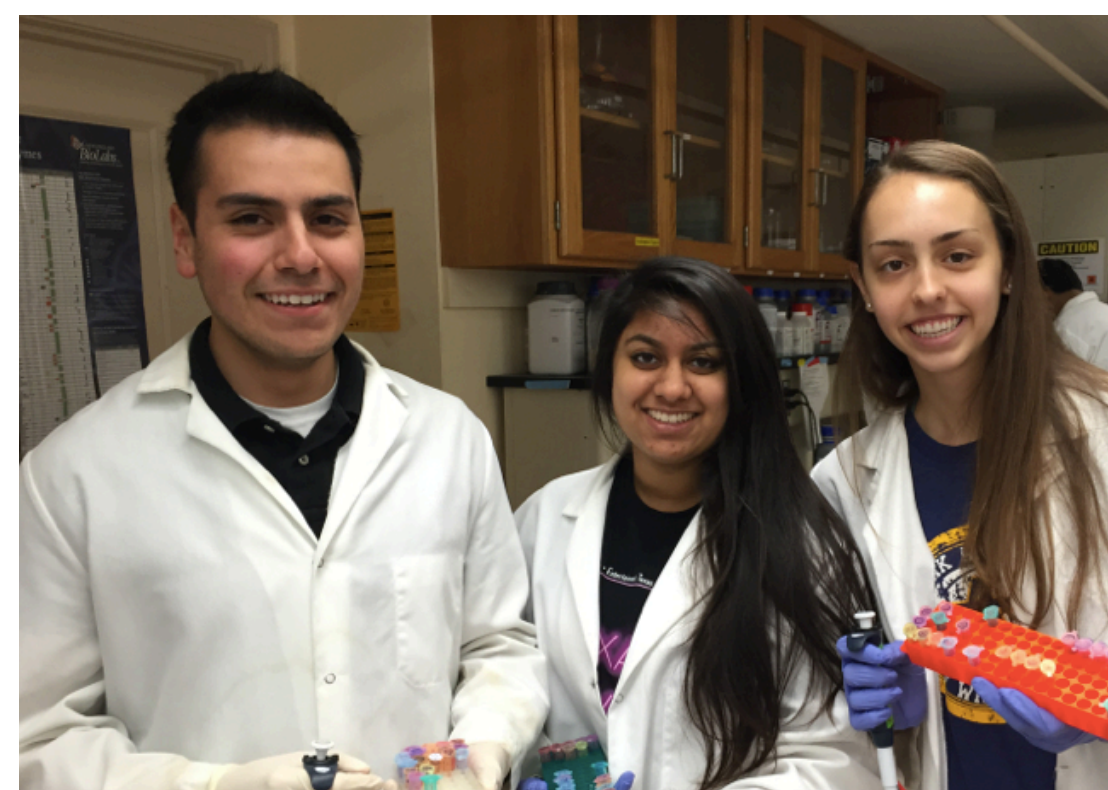
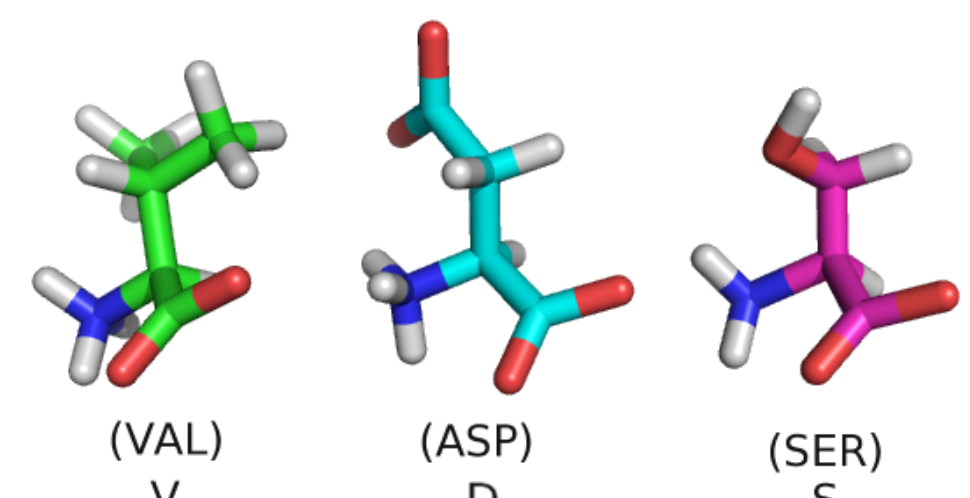


# Virtual Drug Screening (VDS) Stream

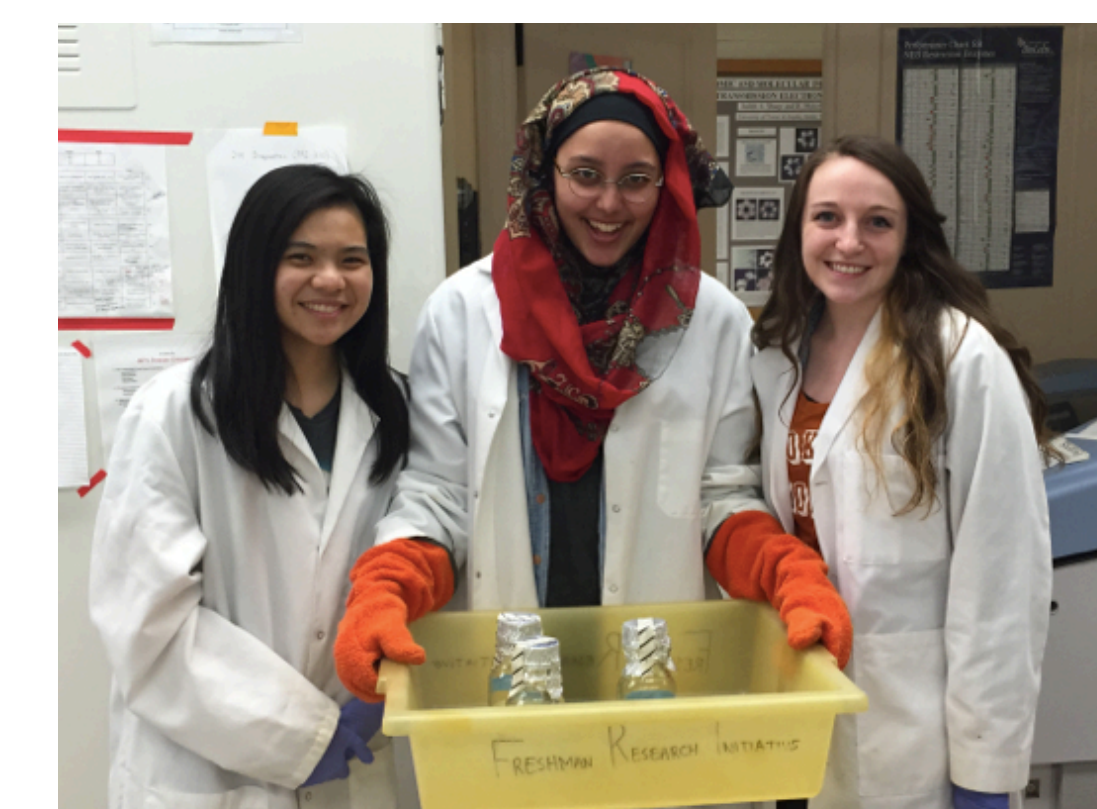
## Finding a Novel Treatment for the Bubonic Plague by Targeting YopH



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### ABSTRACT

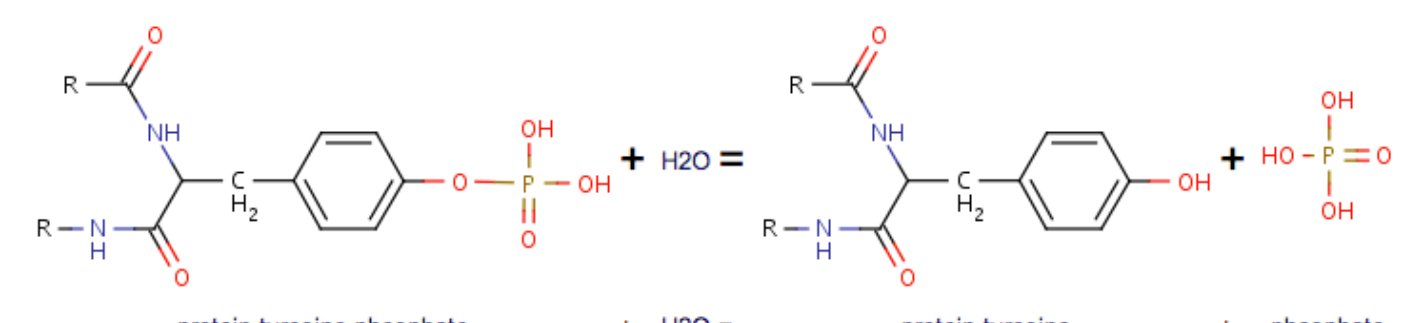
Enzymatic proteins are at the heart of many disease processes. The ability to effectively target and inhibit their molecular function provides an opportunity to mitigate the deleterious outcomes of disease states in humans. However, **identifying new drug leads using traditional methods is an expensive and time consuming process.** This research stream uses **computers to sift through libraries of chemical structures** and predict which ones may bind most effectively to a protein that is a potential drug target. Bubonic plague, caused by *Yersinia pestis*, is a very severe problem in many parts of the world and is the focus of the research this semester for the stream. By targeting YopH, also known as Tyrosine protein specific phosphatase, which is known to be involved in many cellular pathways such as cell growth and cell differentiation, with specific drugs that bind to YopH its function can be reduced and the bacteria hindered, effectively treating the bubonic plague. Through this work students are introduced to fundamental features of protein structures and of protein-ligand interactions. Virtual drug screening software is used and the results are then visualized and interpreted with a molecular graphics program. **Several of the best candidate molecules can then be tested in the wet lab to determine their efficacy in comparison to the computational predictions.**

### INTRODUCTION

#### *Yersinia pestis* and the Bubonic Plague

- Bubonic plague
  - Zoonotic disease caused by *Yersinia pestis*, a gram negative bacteria
  - If left untreated, 3 out of 4 patients die within 4 days of infection
  - Biological weapon threat due to ability to be transmitted by aerosol, high fatality rate and its rapid secondary spread rate
  - Increasing antibiotic resistance
  - Hence the need for novel treatments for the bubonic plague
- YopH
  - Tyrosine specific protein phosphatase
  - Phosphatase that dephosphorylates a tyrosine residue
  - Involved in cell growth, cell differentiation, mitotic cycles and oncogenic transformation

Reaction catalyzed by protein-tyrosine-phosphatase (2.1.3.48)



**Figure 1.** The reaction where Protein Tyrosine Phosphatase is hydrolyzed to form Protein Tyrosine and Phosphate. This reaction usually occurs in the cytosol of cells.

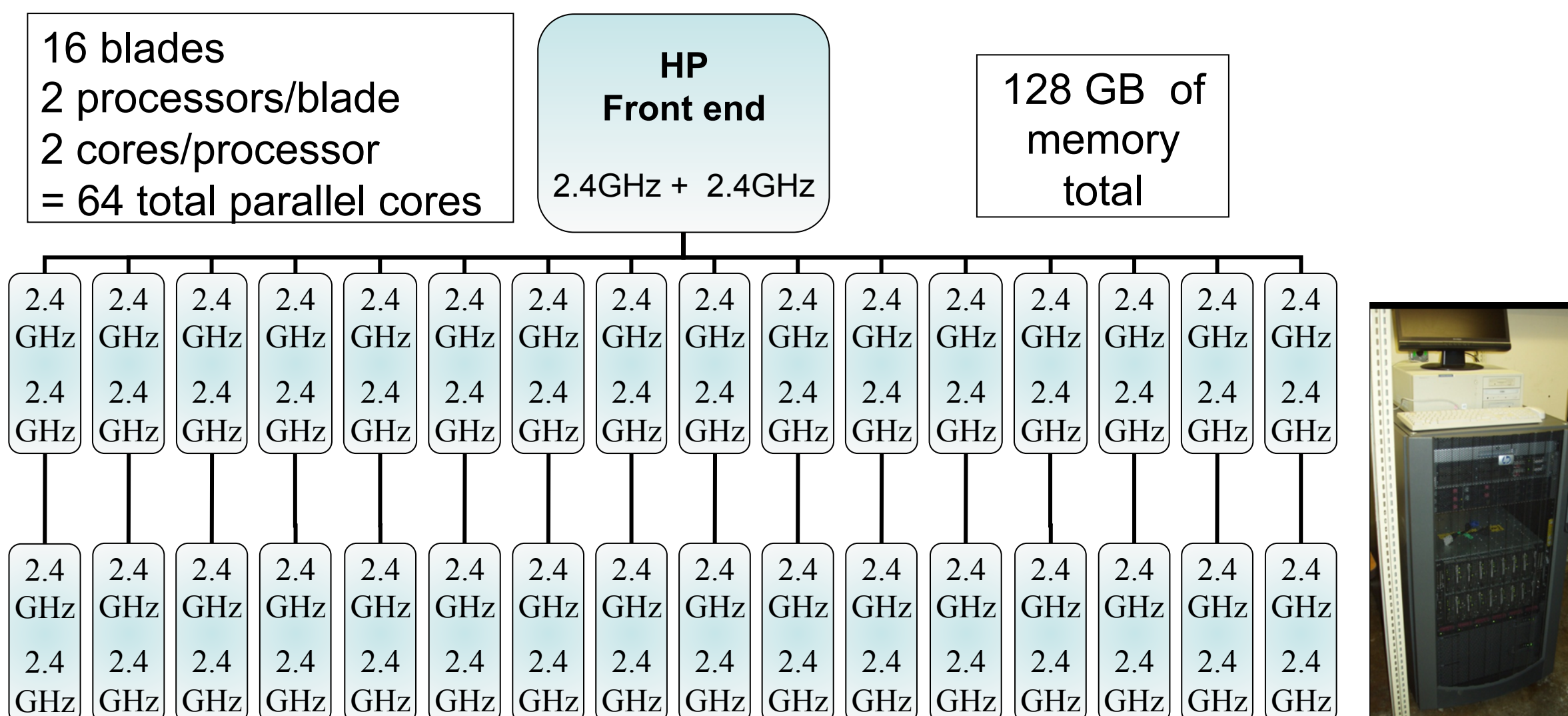
### METHODS

#### Virtual Screening

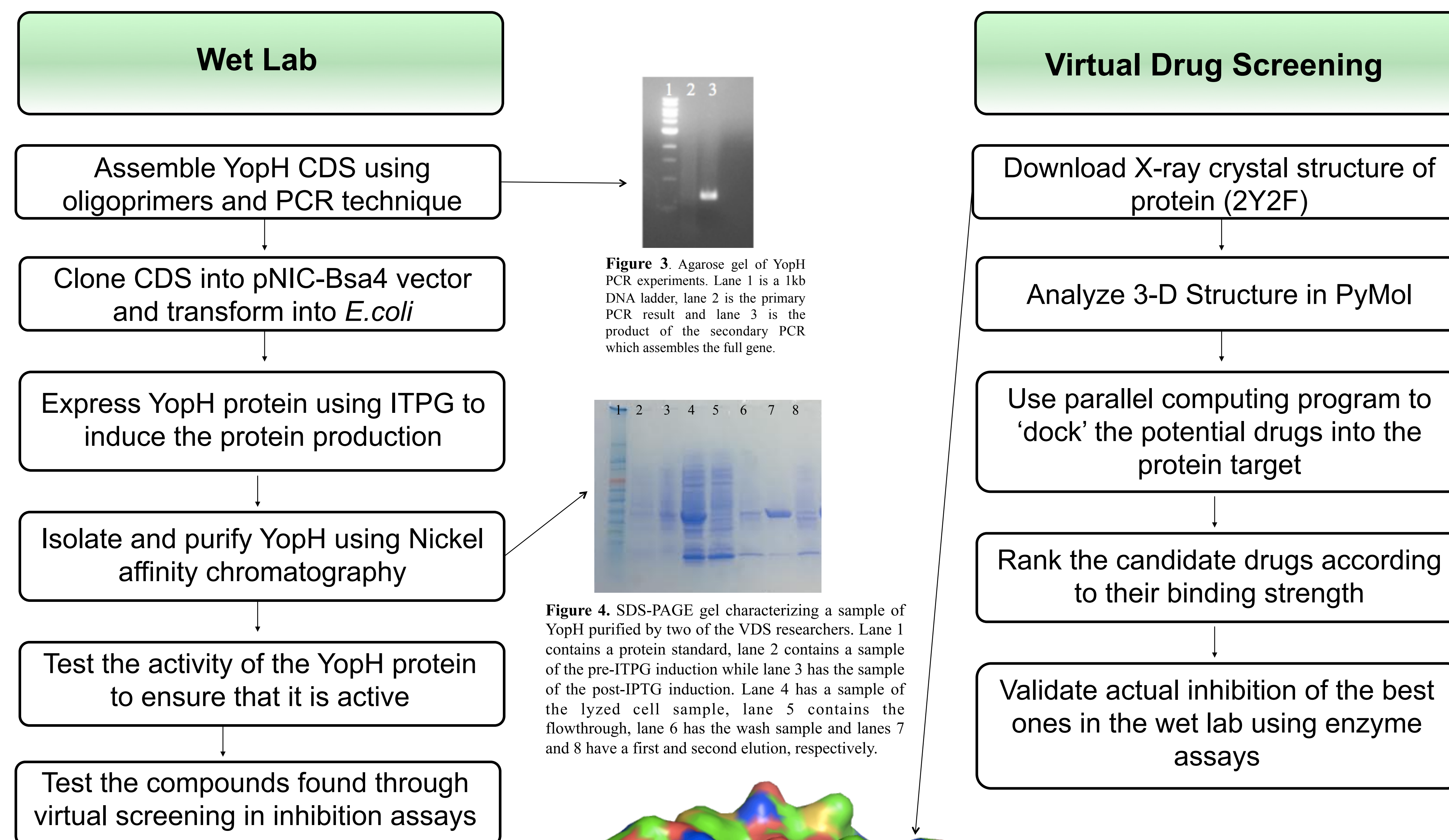
- PyMol<sup>2</sup> was used to visualize the structure of YopH which was taken from the PDB (2Y2F)
- Primary, secondary, and tertiary structure of YopH were analyzed using PyMol and the active site was determined for virtual screening
- Virtual screening was then performed to see if there were any small molecules that could potentially be inhibitors of YopH
- Gold, a program that uses an algorithm to predict protein-ligand binding, was used to screen a small molecule library of 50,000 ligands from ChemBridge

#### Wet Lab

- Assemble the cloning DNA sequence of YopH by using oligoprimers and PCR technique
- Insert the clones DNA into a vector, pNIC-Bsa4 and then transform into *E.coli* DH5alpha
  - This is then sent to sequencing to ensure that desired DNA sequence is present
- The plasmid is then transformed into *E.coli* BL21(DE3) cells for expression
- A large-scale expression is then done to mass produce YopH protein, induced by IPTG
- The YopH protein is then isolated and purified using Nickel affinity chromatography
- Produced protein is then tested for activity using spectrophotometric assays
- After virtual screening produces potential inhibitory compounds, those would be tested for reduced enzymatic YopH activity

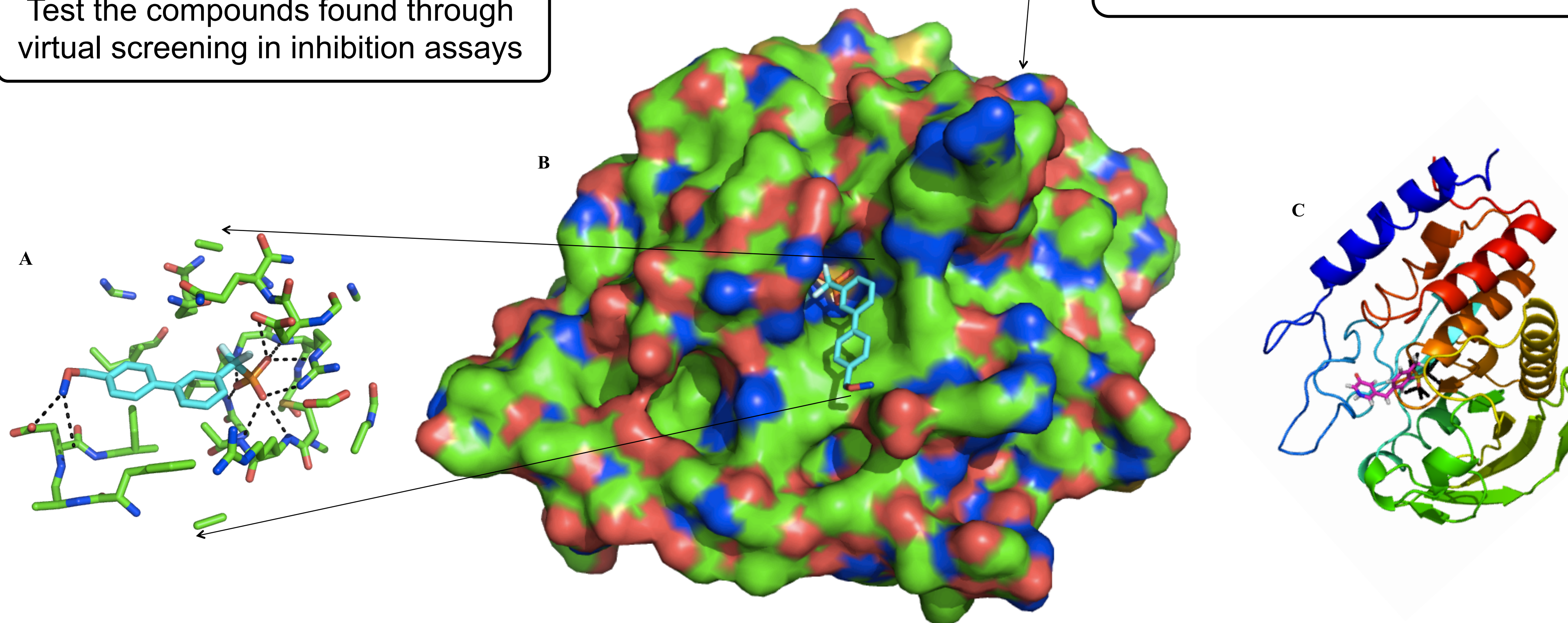


TI3D Cluster

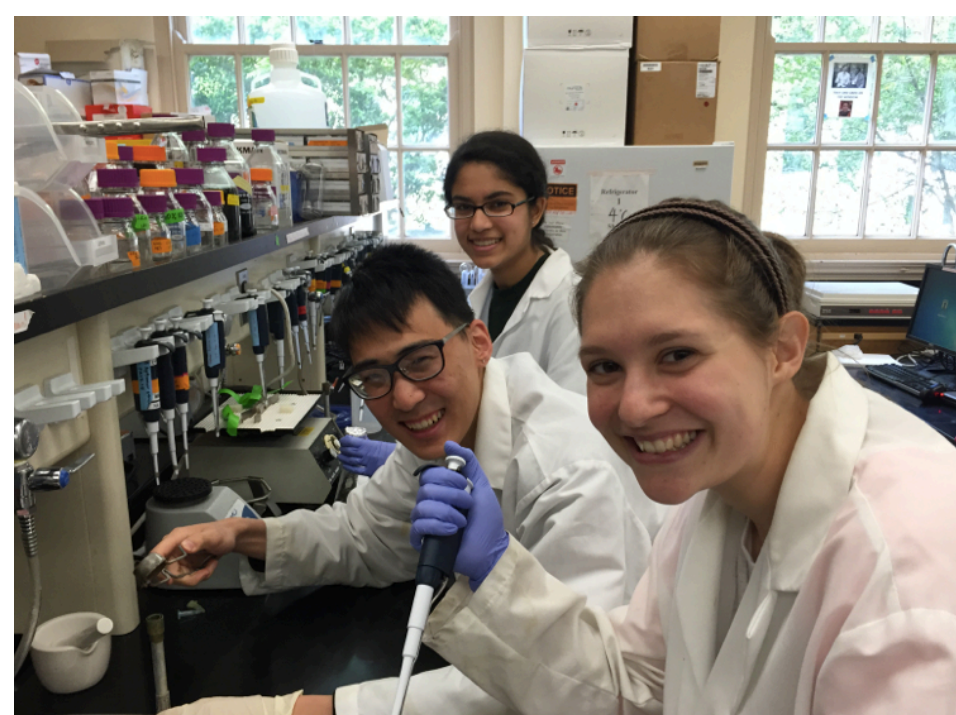


**Figure 3.** Agarose gel of YopH PCR experiments. Lane 1 is a 1kb DNA ladder, lane 2 is the primary PCR result and lane 3 is the product of the secondary PCR which assembles the full gene.

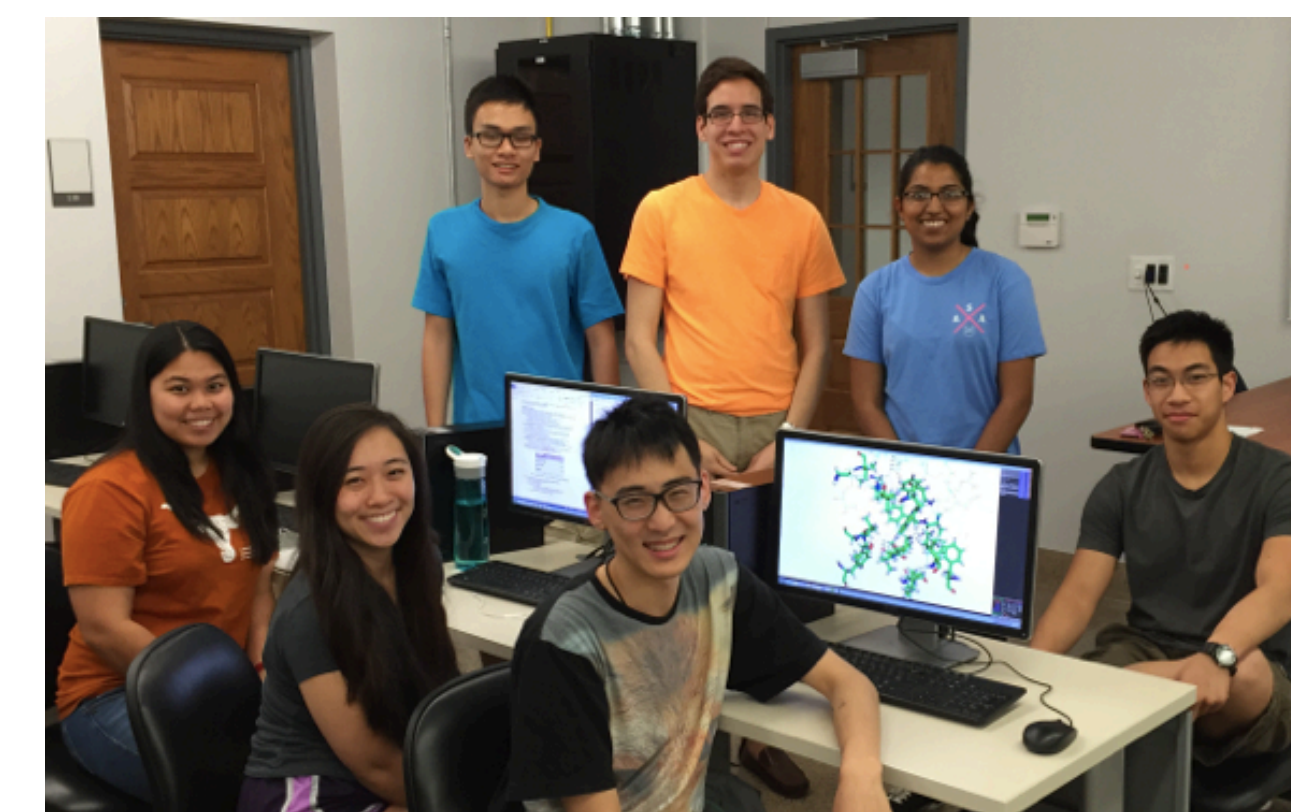
**Figure 4.** SDS-PAGE gel characterizing a sample of YopH purified by two of the VDS researchers. Lane 1 contains a protein standard, lane 2 contains a sample of the pre-IPTG induction while lane 3 has a sample of the post-IPTG induction. Lane 4 has a sample of the lysed cell sample, lane 5 contains the flowthrough, lane 6 has the wash sample and lanes 7 and 8 have a first and second elution, respectively.



**Figure 5:** A) Y11, which was crystallized with 2Y2F, is the natural substrate of YopH and is shown binding to the active site of YopH. The active site was defined to be 7 Angstrom around the Y11 substrate and is shown as green sticks. The Y11 substrate is shown as sticks with carbons colored cyan and the polar interactions between the two are shown as black dashes. B) The PDB structure is shown as a surface with carbons colored green and the substrate in the active site is shown as sticks and colored by element with carbon being cyan. C) Docked in the active site is Chembridge ligand ID # 5852635, a molecule that scored well in virtual screening, shown as sticks colored by element with carbon colored in magenta and the YopH protein is shown as a cartoon colored by chain.



Researchers of the Virtual Drug Screening class



### METHODS (con't)

#### VIRTUAL SCREENING

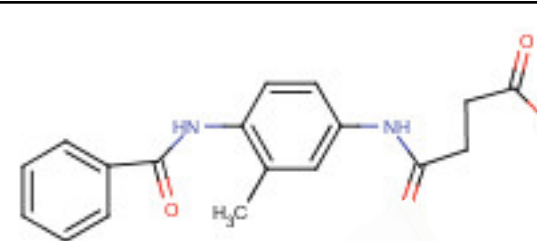
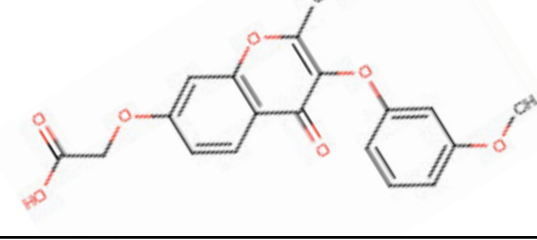
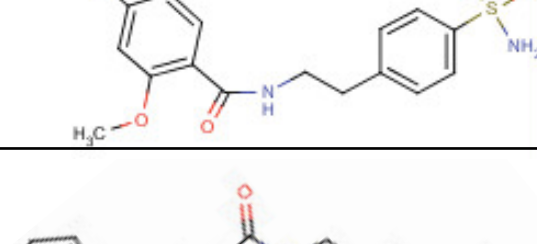
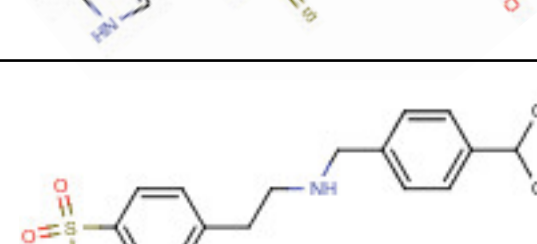

In order to dock each ligand into the three dimensional model of the protein target, a software program called GOLD<sup>4</sup> is implemented in a parallel architecture on the TI3D Drug Discovery Cluster, which contains 16 HP Proliant BL35P blade servers, each with 2 dual core AMD Opteron 2.4 GHz processors for a total of 64 processors (see Fig 3). Each blade contains 8 GB of memory and a 6 GB ATA hard disk drive. The front-end of the cluster is an HP xw9300 Workstation, equipped with a dual core AMD Opteron 2.4 GHz processor, an NVIDIA Quadro FX4500 graphics card, 4 GB memory, and two 500 GB SATA hard drives that is backed up through a RAID5 network.

The GOLD application is capable of scanning through the many different conformations of each ligand that are due to rotatable bonds. For each corresponding docking conformation a Fitness Score is assigned which quantifies the relative strength of the binding of the ligand to the active site of the protein target. This score is an aggregate which incorporates the change in free energies of the binding due to several different chemical interactions: Van der Waals forces, hydrogen bonds, hydrophobic interactions and strain penalties (see Equation below).

$$\Delta G_{bind} = \Delta G_{vdw} + \Delta G_{H-bond} + \Delta G_{hydrophobic} + \Delta G_{rotor} + \Delta G_0$$

### RESULTS

The initial computational screening using GOLD software found the best binding conformation out of 10 different ones for each ligand listed below and gave it a Fitness Score (Table 1). **A higher score corresponds to stronger binding between the enzyme and ligand and, therefore, greater predicted drug activity.** As can be seen from the table below, several compounds gave relatively high GOLD scores, indicating high predicted binding to the active site. A pattern of molecules with two separate benzene groups can be found among the top scoring compounds which is indicative that this might play an important role in the binding of the molecules to the active site.

Name	Score	S(PLP)	S(hbond)	MW	LogP	Hdon	Hacc	Structure
7588592	83.95	-64.63	7.78	374.34	2.3	1	7	
5667642	81.72	-62.94	6.95	356	3.53	1	7	
6588526	81.54	-65.17	6.36	380	1.81	2	5	
5667105	80.93	-66.19	5.43	374	2.82	2	3	
9035543	80.29	-63.88	6.01	369	4.06	2	4	

### CONCLUSIONS

While not providing proof that any compound will inhibit the YopH enzyme *in vivo*, virtual drug screening does help the researcher to **narrow down the field of best possible ligands that could then be tested in the lab**, thereby reducing the time and cost of bringing new drugs to market. The YopH protein was produced successfully in the wet lab and the virtual screening did produce some high scoring potential inhibitors for YopH. The activity of the produced YopH will be tested next along with testing some of the top scoring compounds found through virtual screening to see if they reduce the activity of YopH.

### FUTURE DIRECTIONS (Summer & Fall)

Using the skills from this research stream, students will select other protein targets for virtual screening, such as enzymes involved in bacterial and fungal infections or those used in bioterrorism (like anthrax or ricin). After screening for the best predicted binding, the actual compounds will be obtained commercially for validation in the wet lab using light absorbance based enzymatic assays that quantify the level of inhibition for each drug candidate.

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